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UPLC METHOD DEVELOPMENT AND VALIDATION FOR THE QUANTITATIVE ESTIMATION OF GEFITINIB IN API AND MARKETED PHARMACEUTICAL DOSAGE FORM

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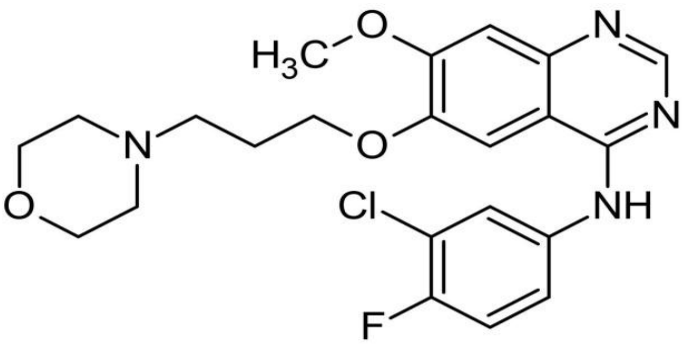
ABSTRACT
An analytical, accurate, precise, specific, efficient and simple Ultra-Performance Liquid Chromatography method has been developed and validated for the determination of Gefitinib in bulk and was applied on marketed Pharmaceutical Dosage form. The mobile phase used for the chromatographic runs consisted of 0.1% OPA Buffer and Acetonitrile in the ratio of 35:65% v/v. The separation was achieved on a BHEL UPLC column using isocratic mode. Gefitinib Drug peak were well separated and were detected by a PDA detector at 254 nm. The developed method was linear at the concentration range 6–14 µg/ml for Gefitinib. The method has been validated according to ICH guidelines with respect to system suitability, specificity, precision, accuracy and robustness. The LOD and LOQ for the Gefitinib were found to be 0.5853 µg/ml and 1.7738µg/ml respectively. The developed method is simple, precise, specific, accurate and rapid, making it suitable for estimation of Gefitinib in bulk and marketed pharmaceutical dosage form dosage form.

KEYWORDS: Gefitinib, UPLC, Accuracy, Precision, Robustness, ICH Guidelines.

INTRODUCTION
Gefitinib is a member of the class of quinazolines that is quinazoline which is substituted by a (3-chloro-4-fluorophenyl) nitrilo group, 3-(morpholin-4-yl) propoxy group and a methoxy group at positions 4, 6 and 7, respectively. An EGFR kinase inhibitor used for the treatment of non-small cell lung cancer. It has a role as an epidermal growth factor receptor antagonist and an antineoplastic agent. It is aromatic ether, a member of Mon chlorobenzenes, a member of mono fluoro benzenes, a secondary amino compound, a tertiary amino compound, a member of quinazolines and a member of morpholines.

Synonyms
Gefitinib, 184475-35-2, Iressa, ZD1839, Irressat, Gefitinibum, CCRIS 9011, UNII-S65743JHBS, Gefitinib (GMP), NSC-759856.

Chemical structure



IUPAC Name
N-(3-chloro-4-fluoro phenyl)-7-methoxy-6-(3-morpholin-4-yl propoxy) quinazolin-4-amine

Molecular formula
$C_{22}H_{24}ClFN_4O_3$

MATERIALS AND METHODS

Chemical and reagents Reference standard of Gefitinib was used to develop the new UPLC method. Acetonitrile was obtained from Sd Fine chem. Ltd (India). Water for UPLC was prepared using Milli Q Water (Merk). Gefitinib HCl is commercially available as Votrient marketed by GSK Rx India with a labeled claim of 200mg per tablet.

Method development
Chromatographic parameters
Equipment: Ultra performance liquid chromatography equipped with Auto Sampler and PDA detector
Column: BHEL UPLC COLUMN
Elution Mode: Isocratic
Flow rate: 0.25 mL per min
Wavelength: 254 nm
Injection volume: 5 µl
Column temperature: Ambient
Run time: 2 min

Preparation of 0.1% OPA Buffer pH-3
To prepare 0.1% OPA buffer solution, by adding 1ml of ortho phosphoric acid in 1000ml water. Adjust this solution to pH 3 by using sodium hydroxide.

Preparation of mobile phase
Mix a mixture of 0.1% OPA buffer 350 ml (35%) and 650 ml Acetonitrile UPLC (65%) and degas in ultrasonic water bath for 5 minutes. Filter through 4.5 µ filter under vacuum filtration.

Diluents preparation
0.1% OPA buffer: Acetonitrile (35:65) ratio.

Wave length selection
UV spectrum of 10µg/ml Gefitinib in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 254 nm. At this wavelength the drug shows good absorbance.

UV Graph

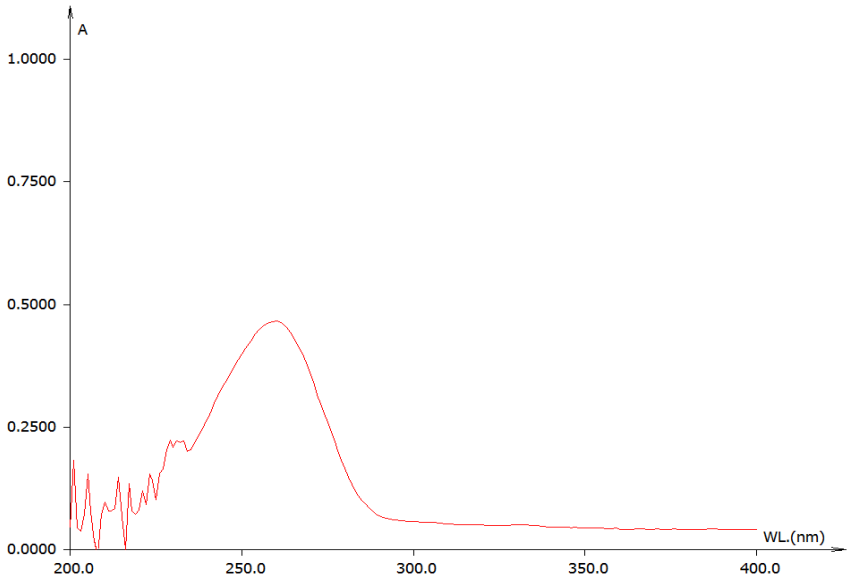


Fig. 1: UV Spectrum of Gefitinib (254nm).

Preparation of the Gefitinib Standard & Sample Solution
Standard solution preparation
Accurately weigh and transfer 10 mg of Gefitinib is taken into a 10ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the diluent. (Stock solution)

Further pipette 0.1ml of Gefitinib of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample solution preparation
Accurately weigh and transfer equivalent to 10 mg of Gefitinib sample is taken into a 10ml clean dry volumetric flask add diluents and sonicate to dissolve it completely and make volume up to the mark with the diluent. (Stock solution)

Further pipette 0.1ml of Gefitinib of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

Procedure

Inject 5 µL of the standard, sample into the chromatographic system and measure the areas for the Gefitinib peaks and calculate the % Assay by using the formula.

Optimized chromatographic conditions

Equipment: Ultra performance liquid chromatography equipped with Auto Sampler and PDA detector

Column: BHEL UPLC COLUMN

Mobile Phase: 0.1% OPA Buffer: Acetonitrile (35:65% v/v)

Flow rate: 0.25 mL per min

Wavelength: 254 nm

Injection volume: 5 µl

Column temperature: Ambient

Run time: 2 min

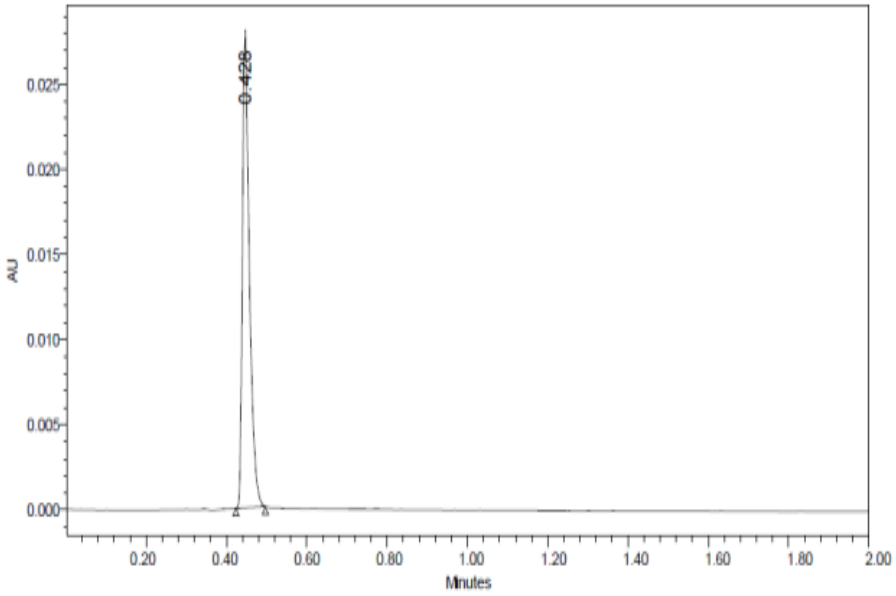


Fig. 2: Optimized chromatogram of gefitinib.

Table 1: Results of optimized chromatogram of gefitinib.

S. No.	Name	Retention Time (min)	Area (µV*sec)	Height (µV)	% Area	USP Plate Count	USP Tailing
1	Gefitinib	0.428	511519	273160	100.00	3559.77	1.34

Method validation

System suitability

- ✓ Tailing factor for the peaks due to Gefitinib in Standard solution should not be more than 2.0**.**
- ✓ Theoretical plates for the Gefitinib peaks in Standard solution should not be less than 2000.

Table 2: Results of system suitability for gefitinib.

S. No.	Peak Name	RT (min)	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Gefitinib	0.435	515867	275841	3652.48	1.36
2	Gefitinib	0.438	516854	275486	3568.75	1.38
3	Gefitinib	0.434	515752	275864	3695.49	1.37
4	Gefitinib	0.436	514986	275684	3745.28	1.39
5	Gefitinib	0.435	515874	275468	3865.42	1.34
6	Gefitinib	0.436	516423	275649	3598.47	1.36
Mean			515959.3			
Std. Dev.			635.8596			
% RSD			0.123238			

Acceptance criteria

- ➢ %RSD of five different sample solutions should not more than 2.
- ➢ The %RSD obtained is within the limit, hence the method is suitable.

Specificity

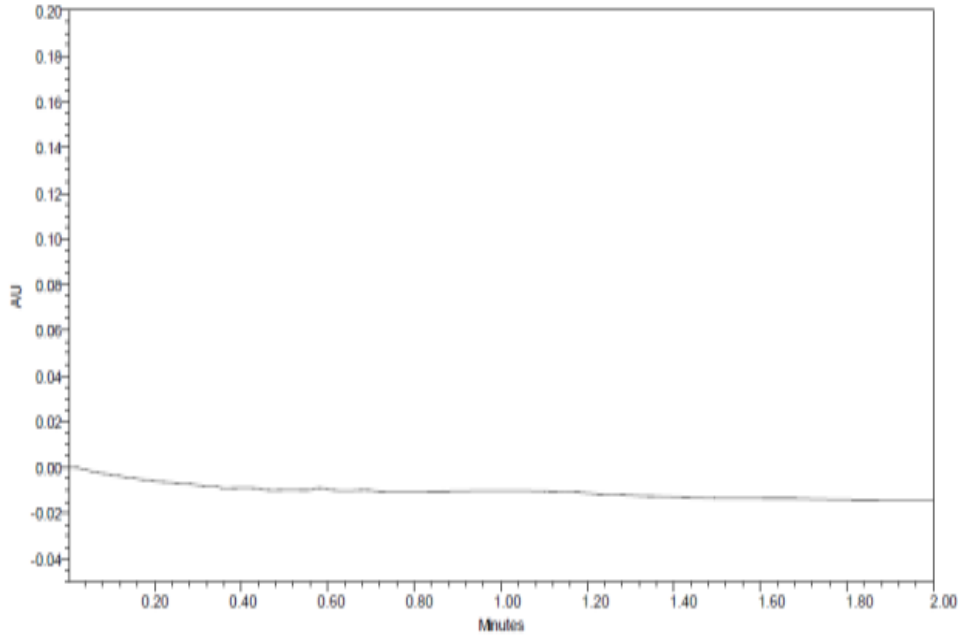


Fig. 3: Blank Chromatogram (Mobile phase preparation).

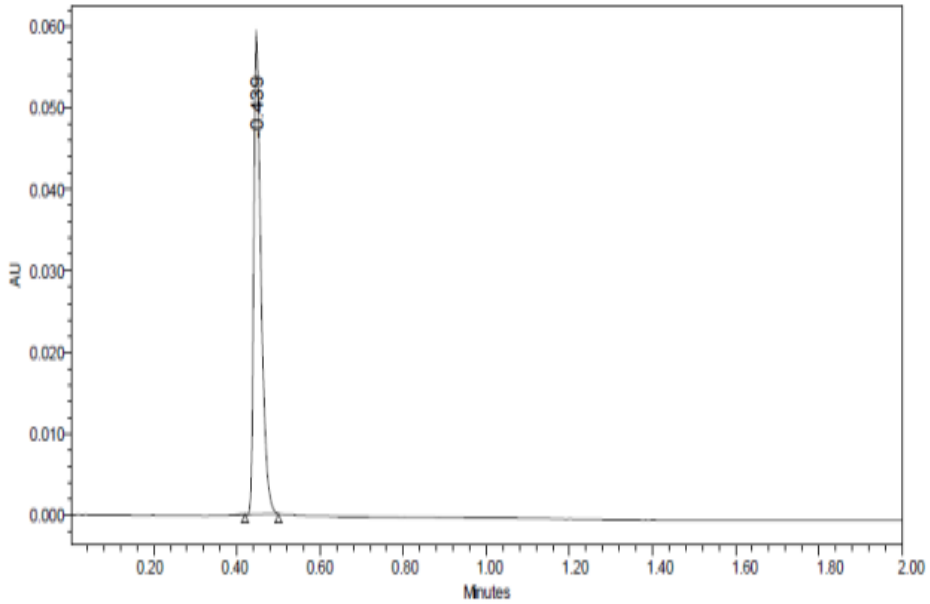


Fig. 4: Standard chromatogram of gefitinib.

Precision

Preparation of stock solution

Accurately weigh and transfer 10 mg of Gefitinib is taken into a 10ml clean dry volumetric flask add diluents and sonicate to dissolve it completely and make volume up to the mark with the diluent. (Stock solution)

Further pipette 0.1 ml of Gefitinib of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure

The standard solution was injected for six times and measured the area for all six injections in UPLC. The %RSD for the area of Six replicate injections was found to be within the specified limits. The results are summarized Gefitinib.

Table 3: Results of repeatability for gefitinib.

S. No.	Peak name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Gefitinib	0.436	516854	273564	3568.69	1.39
2	Gefitinib	0.435	514857	274865	3685.47	1.35
3	Gefitinib	0.436	515863	274981	3598.78	1.37
4	Gefitinib	0.434	516985	278685	3659.84	1.34
5	Gefitinib	0.438	514256	279863	3785.24	3.46
6	Gefitinib	0.435	517854	275258	3692.41	3.47
Mean			516111.5			
Std. Dev			1373.246			
%RSD			0.266075			

Acceptance criteria

- %RSD for sample should be NMT 2.
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Repeatability

Intermediate Precision/Ruggedness

Preparation of stock solution

Accurately weigh and transfer 10 mg of Gefitinib is taken into a 10ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the diluent. (Stock solution)

Further pipette 0.1ml of Gefitinib of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions.

Procedure

The standard solution was injected for six times and measured the area for all six injections in UPLC. The %RSD for the area of six replicate injections was found to be within the specified limits. The results are summarized Gefitinib.

Table 4: Results of intermediate precision for gefitinib.

S. No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Gefitinib	0.438	518659	278543	3658.75	1.39
2	Gefitinib	0.439	518696	275465	3768.45	1.38
3	Gefitinib	0.435	519632	278564	3898.38	1.34
4	Gefitinib	0.434	518748	274584	3785.96	1.38
5	Gefitinib	0.434	519865	274869	3648.74	1.37
6	Gefitinib	0.435	518523	273542	3895.24	1.36
Mean			519020.5			
Std. Dev.			573.5604			
% RSD			0.110508			

Acceptance criteria

The % RSD for the area of six standard injections results should not be more than 2%.

Accuracy

Preparation of standard stock solution

Accurately weigh and transfer 10mg of Gefitinib is taken into a 10ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the diluent. (Stock solution)

Further pipette 0.05ml of Gefitinib of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent

Table 5: The accuracy results for gefitinib.

Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	266301.7	5	5.018	100.360	100.195%
100%	528178.7	10	10.002	100.020	
150%	792391	15	15.031	100.206	

Acceptance criteria
The % Recovery for each level should be between 98.0 to 102.0%.

Accuracy for 50%
Table 6: Results of Accuracy for concentration-50%.

S. No.	Name	RT	Area	Height	USP Plate Count	USP Tailing	Injection
1	Gefitinib	0.434	265864	136582	3869.85	1.38	1
2	Gefitinib	0.436	266898	136487	3798.46	1.39	2
3	Gefitinib	0.428	266143	136825	3988.75	1.37	3

Table 7: Results of Accuracy for concentration-100%.

S. No.	Name	RT	Area	Height	USP Plate Count	USP Tailing	Injection
1	Gefitinib	0.435	527868	284573	3968.45	1.39	1
2	Gefitinib	0.439	528679	286574	3947.46	1.38	2
3	Gefitinib	0.436	527989	285425	3899.96	1.36	3

Accuracy For 150%
Table 8: Results of Accuracy for concentration-150%.

S. No.	Name	RT	Area	Height	USP Plate Count	USP Tailing	Injection
1	Gefitinib	0.435	793574	402546	3986.85	1.41	1
2	Gefitinib	0.439	791245	401698	3989.96	1.43	2
3	Gefitinib	0.436	792354	402857	3979.59	1.46	3

Linearity
Accurately weigh and transfer 10 mg of Gefitinib is taken into a 10ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the diluent. (Stock solution)

Further pipette 0.1ml of Gefitinib of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of stock solution
Accurately weigh and transfer 10 mg of Gefitinib is taken into a 10ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the diluent. (Stock solution)

Preparation of Level – I (6ppm of Gefitinib)
0.06 ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – II (8ppm of Gefitinib)
0.08 ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – III (10ppm of Gefitinib)
0.1 ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – IV (12ppm of Gefitinib)
0.12 ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – V (14ppm of Gefitinib)
0.14 ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Procedure
Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Table 9: Linearity results: (for Gefitinib).

S. No.	Linearity Level	Concentration	Area
1	I	6	323566
2	II	8	421274
3	III	10	528589
4	IV	12	632787
5	V	14	736598
Correlation Coefficient			0.999

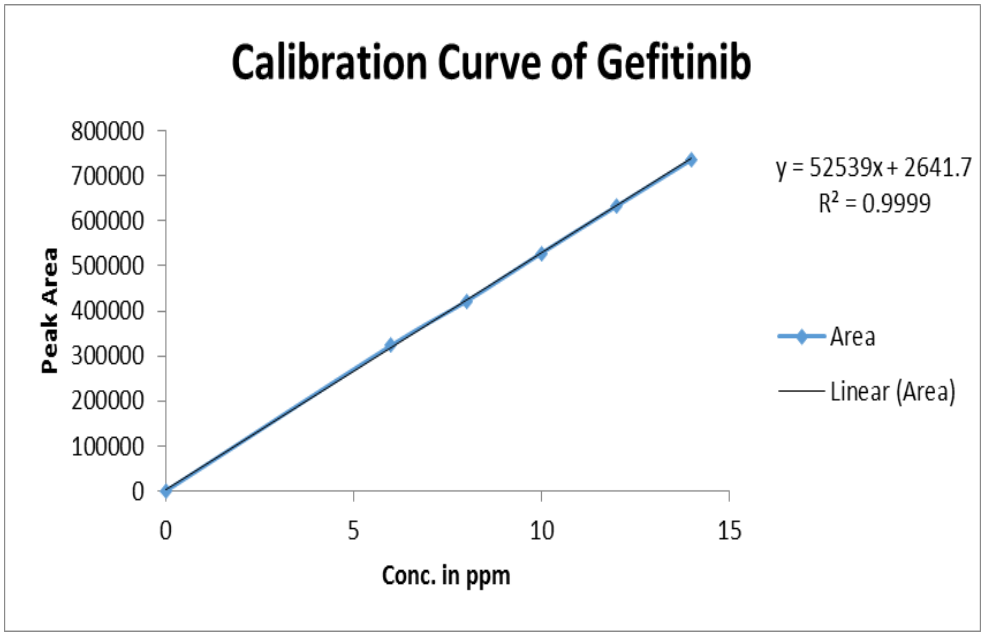


Fig. 5: Calibration curve of gefitinib.

Acceptance criteria
Correlation coefficient should be not less than 0.999.

Robustness
As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition Temperature Variation was made to evaluate the impact on the method.

a) The flow rate was varied at 0.225 ml/min to 0.275 ml/min
Standard solution 10 ppm of Gefitinib prepared and analysed using the varied flow rates along with method flow rate.
The results are summarized
On evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10%.
The method is robust only in less flow condition.

Table 10: System suitability results for gefitinib.

S. No.	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.225	3912.96	1.33
2	0.25	3559.77	1.34
3	0.275	3777.23	1.37

***** Results for actual flow (0.25ml/min) have been considered from Assay standard.

b) The Organic composition in the Mobile phase was varied from 55% to 75%
Standard solution 10µg/ml of Gefitinib was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method.

The results are summarized
On evaluation of the above results, it can be concluded that the variation in 10% Organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is robust even by change in the Mobile phase ±10.

Table 11: System suitability results for gefitinib.

S. No.	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	3456.84	1.50
2	*Actual	3559.77	1.34
3	10% more	3658.61	1.20

LOD and LOQ
LOD: The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.
LOD= 3.3 × σ / s

Where
σ = Standard deviation of the response
S = Slope of the calibration curve
LOQ: The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.
LOQ = 10×σ/S

Observation: On the evaluation of above results the LOD and LOQ for the Gefitinib was found to be 0.5853 µg/ml and 1.7738µg/ml respectively.

Assay of marketed formulation
Gefitero Tablet 250mg
Twenty Tablets were taken and the I.P. method was followed to determine the average weight. Above weighed Tablets were finally powdered and triturated well. A quantity of powder equivalent to 25 mg of drugs were transferred to 25 ml volumetric flask, make and solution was sonicated for 15 minutes, there after volume was made up to 25 ml with same solvent. Then 10 ml of the above solution was diluted to 100 ml with mobile phase. The solution was filtered through a membrane filter (0.45 µm) and sonicated to degas. The solution prepared was injected in five replicates into the HPLC system and the observations were recorded.

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \text{Avg. Wt} = \text{mg}$$

Where:
AT = Peak Area of drug obtained with test preparation
AS = Peak Area of drug obtained with standard preparation
WS = Weight of working standard taken in mg
WT = Weight of sample taken in mg
DS = Dilution of Standard solution
DT = Dilution of sample solution
P = Percentage purity of working standard

RESULT AND DISCUSSION
The %Purity of Marketed Formulation of Gefitinib was found to be 99.536%.

Standard solution

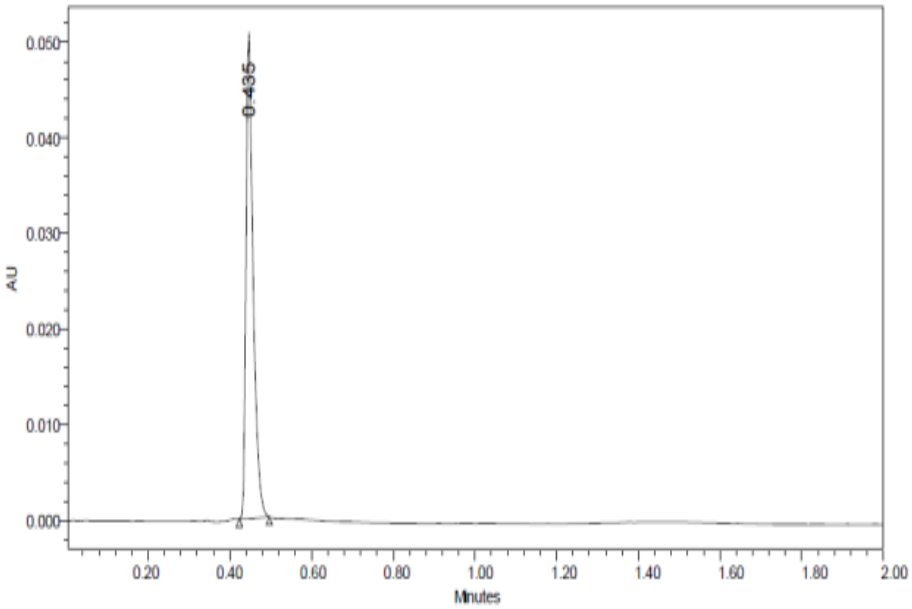


Fig. 6: Chromatogram of standard solution-1.

Table 12: Results of standard solution-1.

S. No.	Name	Retention Time (min)	Area (µV*sec)	Height (µV)	% Area	USP Plate Count	USP Tailing
1	Gefitinib	0.435	511519	273160	100.00	3559.77	1.34

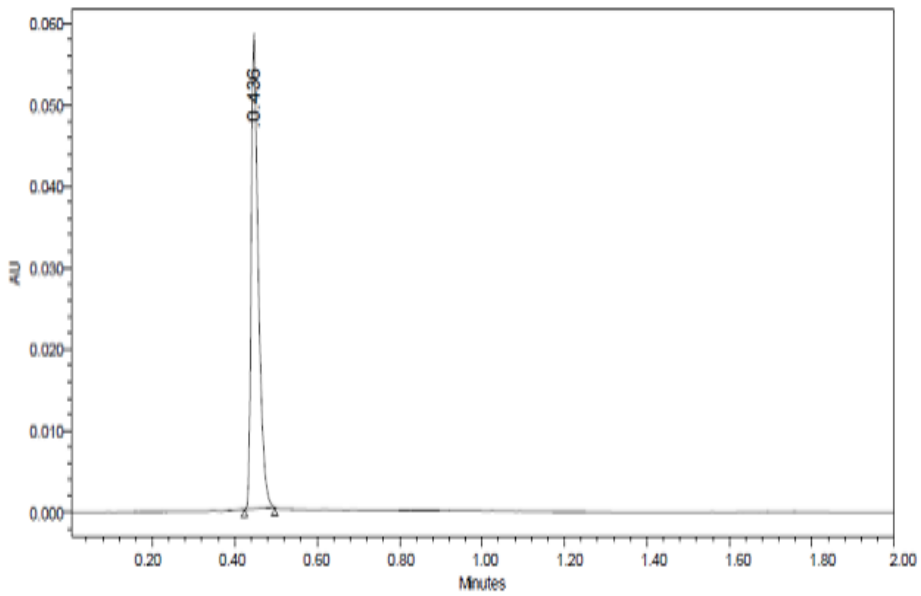


Fig. 7: Chromatogram of standard solution-2.

Table 13: Results of standard solution-2.

S. No.	Name	Retention Time (min)	Area (µV*sec)	Height (µV)	% Area	USP Plate Count	USP Tailing
1	Gefitinib	0.436	515752	275864	100.00	3490.36	1.31

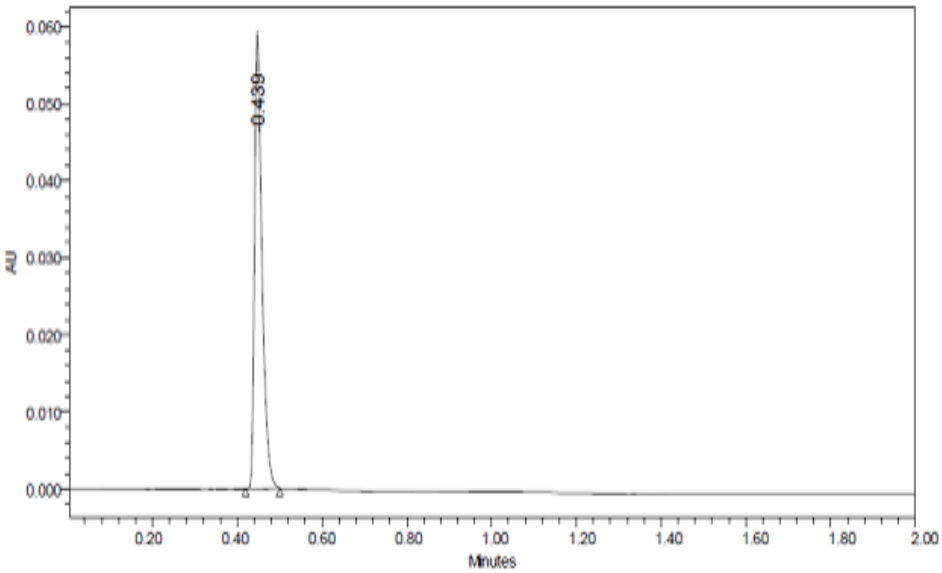


Fig. 8: Chromatogram of standard solution-3.

Table 14: Results of standard solution-3.

S. No.	Name	Retention Time (min)	Area (µV*sec)	Height (µV)	% Area	USP Plate Count	USP Tailing
1	Gefitinib	0.439	518732	277410	100.00	3464.48	1.32

Sample solution

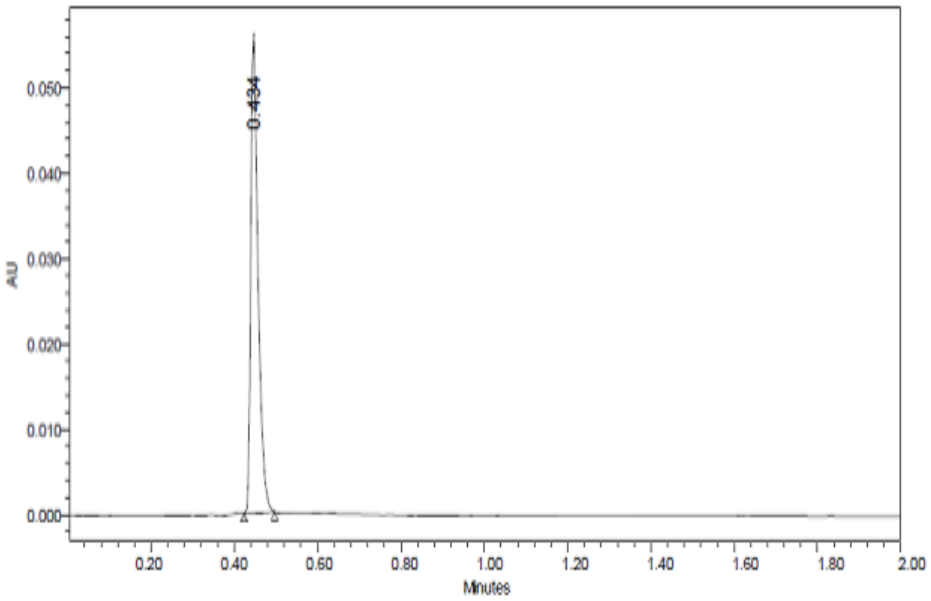


Fig. 9: Chromatogram of sample solution-1.

Table 15: Results of sample solution-1.

S. No.	Name	Retention Time (min)	Area (µV*sec)	Height (µV)	% Area	USP Plate Count	USP Tailing
1	Gefitinib	0.434	511928	302502	100.00	3491.06	1.36

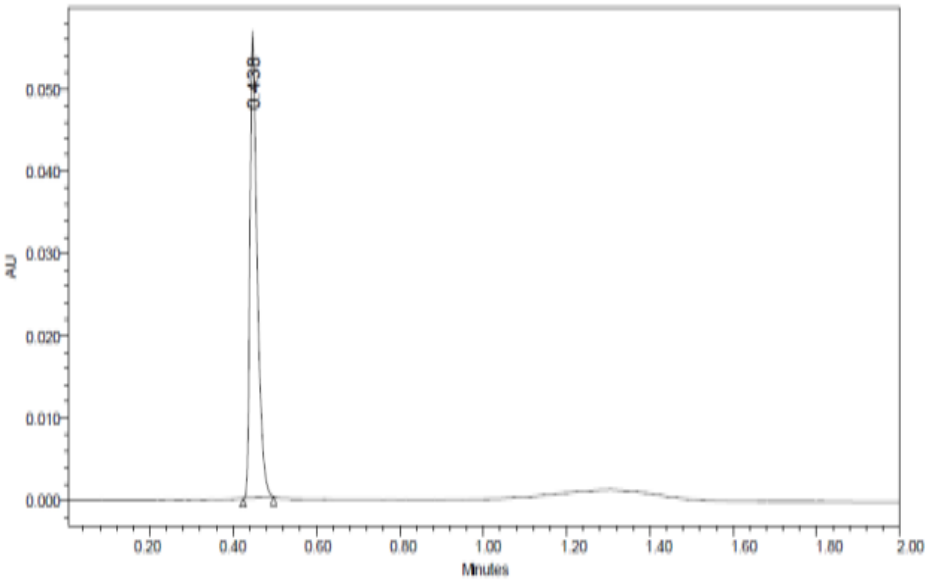


Fig. 10: Chromatogram of sample solution-2.

Table 16: Results of sample solution-2.

S. No.	Name	Retention Time (min)	Area (µV*sec)	Height (µV)	% Area	USP Plate Count	USP Tailing
1	Gefitinib	0.438	512846	303651	100.00	3433.64	1.39

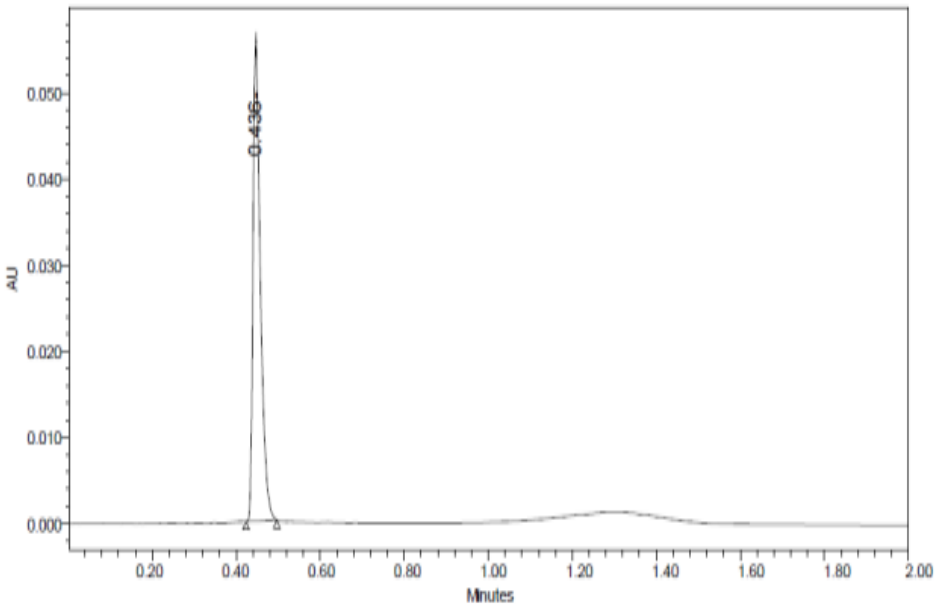


Fig. 11: Chromatogram of sample solution-3.

Table 17: Results of sample solution-3.

S. No.	Name	Retention Time (min)	Area (µV*sec)	Height (µV)	% Area	USP Plate Count	USP Tailing
1	Gefitinib	0.436	514838	305831	100.00	3428.12	1.38

Table No. 18: Results of degradation studies.

Sample Name	Gefitinib	
	Area	% Degraded
Standard	511519	0.000
Acid	496859	2.866
Base	495047	3.221
Peroxide	499327	2.384
Thermal	490258	4.157
Photo	509289	0.436

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate UPLC method was developed for the quantitative estimation of Gefitinib in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatization or purification steps. OPA Buffer: Acetonitrile (35:65% v/v) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for UPLC method was promising. The UPLC method is more sensitive, accurate and precise compared to the HPLC & Spectrophotometric methods. This method can be used for the routine determination of Gefitinib in bulk drug and in Pharmaceutical dosage forms.

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